An efficient method for fractionated whole rodent brain radiation

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Abstract: OBJECTIVE: In order to test for mechanisms of whole brain radio therapy side effects and possible neuroprotective measures, a rodent model is desirable. In many models, a high single dose of 8-20 Gray (Gy) of whole brain irradiation is used. These experimental radiation protocols do not closely reflect the clinical situation, where the cumulative dosage is applied in smaller fractions. We describe an efficient method to perform repetitive, fractionated whole brain radio therapy to the rat brain. METHODS: Fifteen-week-old rats were irradiated with a dose of 5 or 10 Gy on four consecutive days, resulting in a cumulative dose in opposing fields of 20 Gy (n = 15) and 40 Gy (n = 17), respectively. Sham-irradiated rats (n = 14) received the same procedure but without application of cranial irradiation. Four collimators with a diameter of 3 cm each were used to place four rats and an ionization chamber simultaneously in the dose field for monitoring. RESULTS: Fourteen days after the procedure, irradiated animals showed decreased open-field activity (two-tailed t-test, sham versus 20 Gy, $P<0.001$; sham versus 40 Gy, $P = 0.002$), but no cognitive deficit as indicated by latencies in the Morris water maze test. Six weeks after the irradiation, no group showed alterations of histopathology such as vascular changes, demyelination, or white matter necrosis. DISCUSSION: The proposed model represents an efficient and safe method to perform fractioned high-dose irradiation of the rodent brain. Speculatively, it is possible to increase the cumulative dosage and dose per fraction used in this model to achieve a higher degree of radiation-induced toxicity.

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Objective: In order to test for mechanisms of whole brain radio therapy side effects and possible neuroprotective measures, a rodent model is desirable. In many models, a high single dose of 8–20 Gray (Gy) of whole brain irradiation is used. These experimental radiation protocols do not closely reflect the clinical situation, where the cumulative dosage is applied in smaller fractions. We describe an efficient method to perform repetitive, fractionated whole brain radio therapy to the rat brain.

Methods: Fifteen-week-old rats were irradiated with a dose of 5 or 10 Gy on four consecutive days, resulting in a cumulative dose in opposing fields of 20 Gy ($n=15$) and 40 Gy ($n=17$), respectively. Sham-irradiated rats ($n=14$) received the same procedure but without application of cranial irradiation. Four collimators with a diameter of 3 cm each were used to place four rats and an ionization chamber simultaneously in the dose field for monitoring.

Results: Fourteen days after the procedure, irradiated animals showed decreased open-field activity (two-tailed *t*-test, sham versus 20 Gy, $P<0.001$; sham versus 40 Gy, $P=0.002$), but no cognitive deficit as indicated by latencies in the Morris water maze test. Six weeks after the irradiation, no group showed alterations of histopathology such as vascular changes, demyelination, or white matter necrosis.

Discussion: The proposed model represents an efficient and safe method to perform fractioned high-dose irradiation of the rodent brain. Speculatively, it is possible to increase the cumulative dosage and dose per fraction used in this model to achieve a higher degree of radiation-induced toxicity.

Keywords: Method report, Rodent model, Whole brain radio therapy

Introduction

Whole brain radio therapy (WBRT) is used to treat patients with primary or metastatic brain tumors or as prophylactic treatment in adult patients with small cell lung cancer or pediatric patients with acute lymphocytic leukemia. To minimize the side effects of WBRT, the whole dose is generally administered in fractions of 3 Gray (Gy) or less. Despite that, complications after WBRT such as cognitive deficits are common, especially in long-term cancer survivors.[1,2] Cranial irradiation of rodents shows that the long-term cognitive deficits are associated with impairment of hippocampal neurogenesis and an activation of microglia.[3–6] Due to the higher resistance of rodent brain to whole brain radiation,[7–9] most studies applied a high (8–15 Gy) single dose. These experimental radiation protocols do not closely reflect the clinical situation, where WBRT is applied in smaller fractions. In addition to that, a dose of 10 Gy irradiation to rat brain is too low for important irradiation-induced side effects, such as vascular changes, white matter changes, or radionecrosis.[7,10] In order to test for mechanisms of irradiation side effects and possible neuroprotective measures, a rodent model for fractionated low-dose WBRT is highly desirable. We herewith describe an efficient method to perform repetitive, fractionated WBRT to the rat brain.

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Methods

Animals and cranial irradiation

Female Wistar rats (Charles River, Sulzfeld, Germany) weighing 250–300 g were housed in groups of four under standard conditions at a temperature of 22°C (±1°C) and a 12-hour light–dark cycle (light from 6 a.m. to 6 p.m.) with free access to food and tap water.

For cranial irradiation, 15-week-old female rats were anesthetized with a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg). Rats were

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irradiated on a Mevatron MD2 (6 MV; Siemens, Munich, Germany) with a single dose of 5 or 10 Gy on four consecutive days, resulting in an accumulative dose in opposing fields of 20 Gy ($n=15$) and 40 Gy ($n=17$), respectively. The dose prescribed to the clinical target volume (CTV) was identical to the whole brain. The point of prescription was the geometric center of the brain. Sham-irradiated rats ($n=14$) received the same procedure but without application of a cranial irradiation. Four collimators with a diameter of 3 cm each were used to place four rats and an ionization chamber simultaneously in the dose field for monitoring (PinPoint 0.016 cm^3; PTW, Freiburg, Germany). The lead shielding (7 cm thick) was used to limit radiation exposure to structures outside the brain, such as the eyes. Irradiation doses and dose gradient within the four irradiation fields, at the eyes and at the middle of the bodies, were measured with *in vivo* dosemeters using RPL-dosimetry with a GD-302M (Asahi Techno Glass Co Dosemeter, Osaka, Japan).[11] In order to irradiate only the cerebrum, a film was exposed initially and further on a portal image to extract the eyes from cerebrum. The irradiation was performed utilizing opposed fields technique with the rat midline as isocenter plane. Three prominent places were chosen for the dosemeters: eye, in-field, and body.

Examination for lens opacity

Four weeks after irradiation, all animals were examined for radiation cataract (lens opacity) with a conventional slit lamp using the following techniques: observation by optical section with a narrow slit and retroillumination.

Behavioral testing

Open field

Open-field experiments were performed 14 days after irradiation in a silent dimly lit room. The open field was a square acrylic glass box ($60 \times 60 \times 60$ cm). A video camera was placed 200 cm above the center of the open field. Illumination density at the center of the maze was below 1 lux. For data analysis, the ground floor of the box was subdivided by 2×2 lines in nine equally spaced squares. Open-field movements were recorded by computer-aided tracking (v4.02; Chromotrack, San Diego, CA, USA). The following parameters were analyzed: line crossings (horizontal activity), time spent in a 5-cm corridor along the maze walls, time spent in the center square (30×30 cm), and time spent in the four corner squares (10×10 cm) of the maze. Each trial lasted 5 minutes. After each trial, the maze was cleaned thoroughly to avoid confounding effects by urination and fecal boli.

Morris water maze

The task was carried out 14 days after irradiation. The Morris water maze is a circular water tank (120 cm; 40 cm in depth). The tank was filled with water to a height of 25 cm at $\sim 22^{\circ}C$ made opaque by the addition of milk powder.

The escape platform, made of white Plexiglas, had a diameter of 10 cm and was height-adjustable. Swimming paths were stored and analyzed *post hoc* with the system used for the open-field sessions. The room was illuminated by ceiling lamps. A number of obvious distal cues were available during water maze testing including doors, racks, and ceiling texture. During a habituation trial with no platform placed in the pool, the animals had to swim for 60 seconds on the first day of Morris water maze testing. Beginning on the following day, the rats were tested in the place version of the task for 4 days ('place learning', days 1–4). For each animal, the platform was submerged 0.5 cm beneath the water surface in one of the four quadrants of the maze, where it remained during all place learning trials. Rats were placed into the maze from four equally spaced points along the perimeter of the pool. The sequence of entry points was chosen randomly. Every day each rat received six consecutive trials (cutoff, 60 seconds). After reaching the platform, the animals were allowed to stay on it for 30 seconds. If an animal failed to escape within 60 seconds, it was placed onto the platform for 30 seconds. During the 60-second intertrial interval, the animals were placed into a resting cage beside the pool. On a subsequent trial in which the platform was removed from the pool ('spatial probe' or extinction trial, day 10), the mice had to swim again for 60 seconds with no opportunity to escape. We recorded the time the animals spent in the quadrant of the tank where the platform was during the earlier trials.

Processing of brain tissue

Six weeks after whole brain irradiation, animals were killed by an overdose of thiopentone (50 mg/kg). One milliliter of blood was collected by cardiac puncture and stored in ethylenediaminetetraacetic acid-containing vials. Before brain removal, animals were transcardially perfused with phosphate-buffered saline buffer and subsequently with a fixative containing 10% formaldehyde, 10% acetic acid, and 80% methanol, followed by immersion of the brain in the fixative for additional 72 hours at room temperature, and paraffin embedding using standard protocols.

All experiments were carried out in accordance with the animal welfare guidelines and laws of the Federal Republic of Germany and were approved by the local committee.

Histochemistry

For histochemistry, 7-μm thick coronal sections of paraffin-embedded brain were made using a Leica

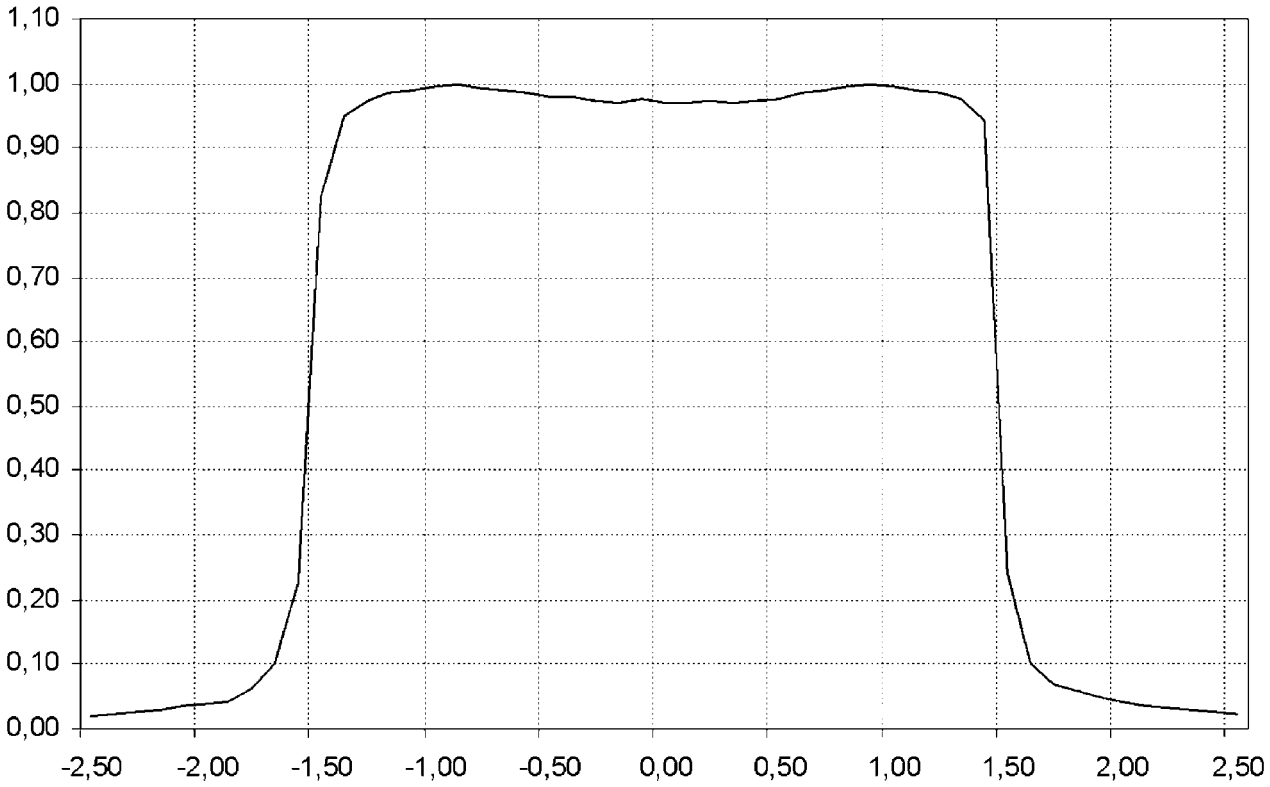

Figure 1 Profile of dose distribution (Gy/mm) over the 3-cm collimators measured with a pin-point device (#31015; PTW, Freiburg, Germany) indicating a penumbra of <4 mm.

microtom model 2155 and mounted on poly-L-lysine-coated slides. Ten consecutive sections were prepared at the following four positions: lateral 0.40 mm, lateral 1.90 mm, lateral 3.20 mm, and 4.32 mm,[12] resulting in 40 representative sections for each animal. Of every group irradiated at 20 or 40 Gy or sham-irradiated animals, five animals were analyzed. All sections were stained with hemotoxylin and eosin according to a standard protocol.

Statistics

For the analysis of behavioral and neurochemical data, *t* tests (two-tailed) for independent and dependent samples were used. Open field and water maze data were analyzed with ANOVA procedures.

Results

Cranial radiation

The cumulative cranial irradiation dose was 19.68 ± 2.83 Gy for the 20 Gy group, and 38.61 ± 5.82 Gy for the group of rats, where a 40 Gy dose was intended. The irradiation dose at the eyes was 1.18 ± 0.38 Gy for the 20 Gy group, and 2.55 ± 0.89 Gy for the 40 Gy group. The cumulative body irradiation dose was 0.98 ± 0.56 Gy (20 Gy cranial irradiation) and 1.39 ± 0.97 Gy (40 Gy). The profile of dose distribution (Gy/mm) over the 3-cm collimators measured with a pin-point device indicated a penumbra of <4 mm (Fig. 1). One rat with a 40-Gy cranial irradiation developed a radiation cataract (lens opacity). All other animals had no lens opacities.

Behavior

Open-field activity expressed as line crossings during the whole 5-minute exposure (total line crossings) showed a difference among all three groups (ANOVA, $P<0.001$). The sham-irradiated animals showed the most line crossings with a significant difference between the sham group and the two irradiated groups (two-tailed *t*-test, sham versus 20 Gy, $P<0.001$; sham versus 40 Gy, $P=0.002$), but no difference between the two irradiation groups (20 Gy versus 40 Gy, $P=0.169$; see Fig. 2).

All groups habituated to the environment (two-tailed *t*-test, line crossings minute 1 versus minute 5, resulting *P* values <0.001; Fig. 2).

In the Morris water maze, all groups learned to find the hidden platform (ANOVA for repeated measures, $P<0.01$), without differences between groups (ANOVA, $P>0.1$). Water maze data thus did not indicate an effect of WBRT on cognition (Fig. 3).

Histopathology

No histopathological changes were detected. No group showed vascular changes, demyelination, or white matter necrosis.

Discussion

Here we report an effective and safe method to perform fractioned high-dose whole rodent brain irradiation. It was possible to apply the intended dose of irradiation only to the cerebrum. The profile of

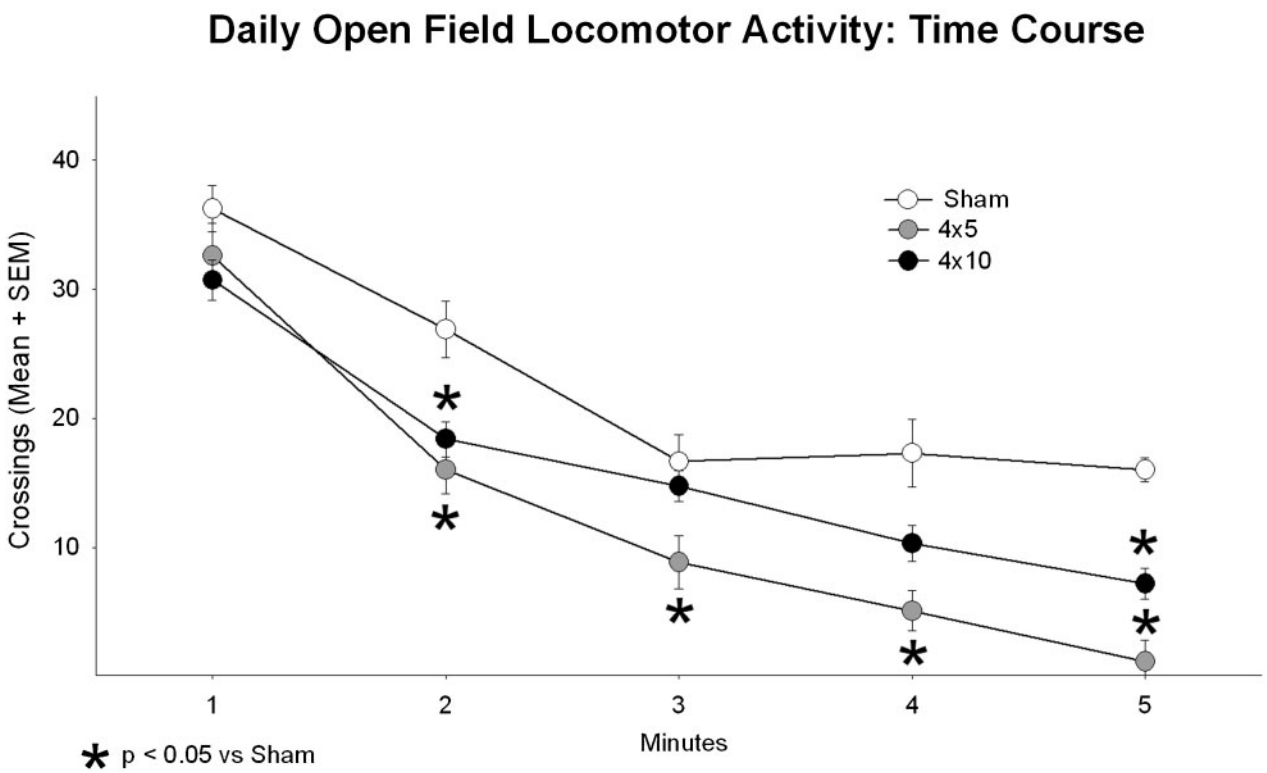

Figure 2 Open-field activity expressed as line crossings during the whole 5-minute exposure (total line crossings) of sham-irradiated animals (sham), animals irradiated with 4×5 Gy (4×5), and animal irradiated with 4×10 Gy (4×10). The sham-irradiated animals showed significantly more activity than the two irradiated groups (sham versus 20 Gy, $P<0.001$; sham versus 40 Gy, $P=0.002$). There was no difference between the two irradiation groups.

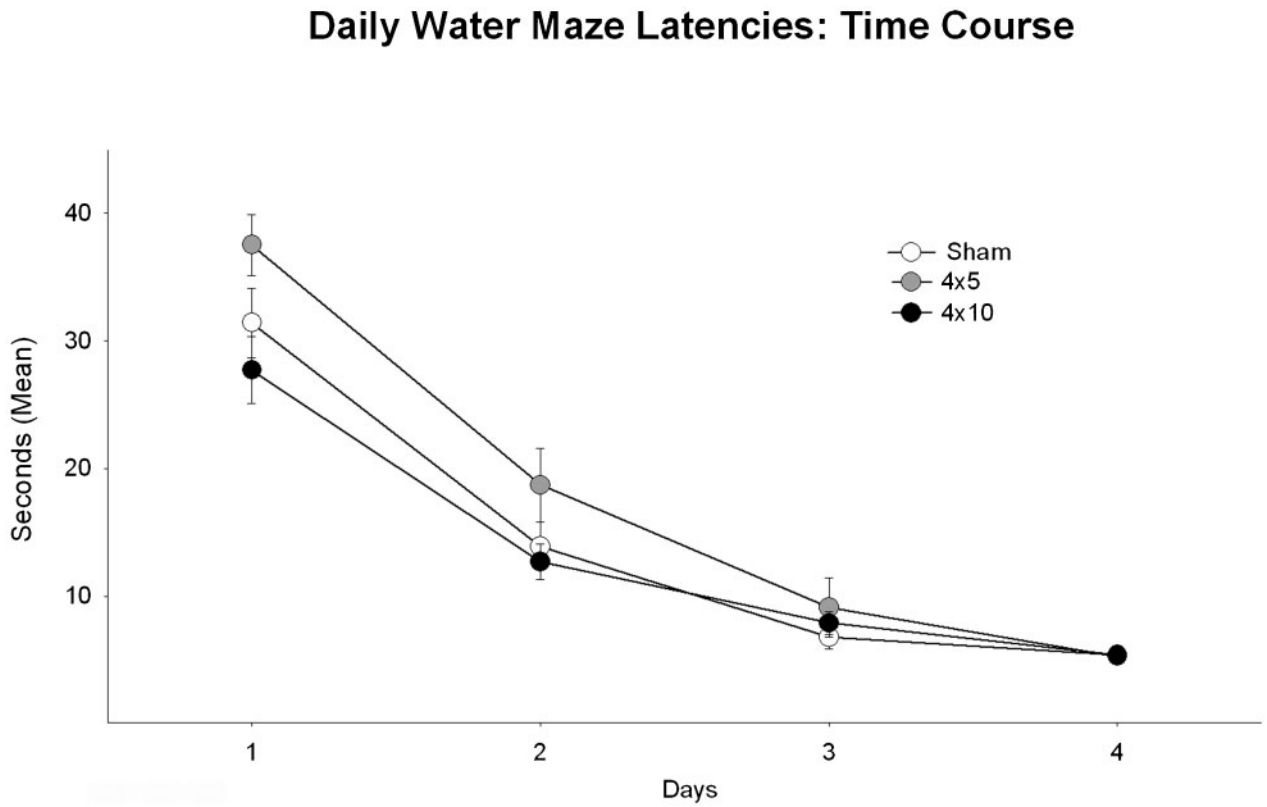

Figure 3 In the Morris water maze, all groups learned to find the hidden platform (ANOVA for repeated measures, $P<0.01$), without differences between groups (ANOVA, $P>0.1$).

dose distribution indicated a penumbra of <4 mm, and only one animal showed a radiation cataract (lens opacity). The parallel radiation of four animals allowed for rapid whole brain radiation of a large number of animals in a short period of time. The high cumulative dosage of 20 or 40 Gy, respectively, did not lead to induction of necrosis, vascular changes, or demyelination on standard histopathological examination, although application of single dose WBRT causes dose-dependent changes, mainly white matter necrosis.[7,10] Fractionated WBRT however, did not lead to changes on histopathology 6 weeks after WBRT. Both dosages (20 and 40 Gy) led to reduced open-field activity 14 days after WBRT. Cranial irradiation did not decrease cognitive performance as measured by the water maze latencies. Thus, reduced open-field activity 4 weeks after WBRT in the rodent model may correspond to the somnolence syndrome seen in patients 1–6 months after WBRT. The somnolence syndrome usually spontaneously resolves within a few weeks and does not indicate patients with an increased risk for late cognitive effects.[13] Cognitive impairment, on the other hand, may become evident as a late effect of WBRT. The late delayed effects of WBRT on cognition and histopathology are detectable around 6–12 months post-irradiation,[14] and have not been tested in the present study.

In summary, this efficient method allows delivering a high cumulative dose of WBRT without induction of acute injury. Speculatively, it is possible to increase the dosage used in this model, to more closely reflect the cumulative dose of up to 60 Gy, which is targeted in the clinical situation, although this is done in smaller fractions of 2 Gy.

The main factors influencing radiation-induced toxicity are the volume of the treated tissue, the total radiation dose, and the fractionation schedule. Brain is especially sensitive to dose per fraction. In a 1970 study, patients received 10 Gy WBRT in one fraction, and 7% of the patients died within 48 hours.[15] Today a fraction size of <2.5 Gy is used. The concept of the biologically effective dose (BED) using the LQ-Model is a useful tool for intercomparing conventional fractionations.[16] With a fraction size of <2.5 Gy, an incidence of radiation necrosis of 5% is expected at a biologically effective dose of 120 Gy (range 100–140 Gy), and 10% radiation necrosis is predicted to occur at 150 Gy (range 140–170 Gy). For fraction sizes larger than 2.5 Gy, the incidence and severity of toxicity is considered to be unpredictable.[17,18] In this experiment, we used four fractions of 5 Gy each, which is equivalent to 53 Gy BED, and four fractions of 10 Gy each, which is equivalent to 173 Gy BED. In the clinical situation, a BED between 60 and 100 Gy is targeted for WBRT. The rat brain however is considerably less sensitive to radiation toxicity and dose per fraction than the human brain.[7,10] Although it is difficult to assign the human toxicity data to rat brain, the rat model should therefore use at least the same BED to achieve comparable radiation-induced toxicity.

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